PATENT
File No.: 98-60C1

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants	:	Zeren Gao et al.
Serial No.	:	09/541,752
Filed	:	March 31, 2001
For	:	ANTIBODIES TO GROWTH FACTOR HOMOLOG ZVEGF3
Examiner	:	Spector, L.
Art Unit	:	1647
Docket No.	:	98-60C1
Date	:	July 2, 2003

Commissioner for Patents
P.O. Box P.O. Box 1450
Alexandria, VA 22313-1450

Declaration of Henry Francis Pelto III Under 37 C.F.R. § 1.132

Sir:

I, Henry Francis Pelto III, do hereby declare as follows:

1. I am currently employed by ZymoGenetics, Inc., the assignee of the above-named patent application, as Research Associate, Protein Biochemistry.

2. I received a Bachelor of Science degree in Biology from Gonzaga University in May 2002.

3. I have read the Office Action mailed March 14, 2003 in the above-identified patent application ("the Patent Application"), including the rejections under 35 U.S.C. §§ 102(e) and 103(a). I am providing this Declaration to assist the patent examiner in evaluating the teachings of Ferrara et al., U.S. Patent No. 6,391,311.

4. I performed an experiment to test the ability of antisera raised against a full-length zvegf3 protein to recognize different forms of zvegf3 (also known as

PDGF-C). The antisera, designated "E2243", was raised in a rabbit by immunization with a full-length human zvegf3 polypeptide fused to *E. coli* maltose binding protein (MBP) and affinity purified using the fusion protein.

5. The experiment was carried out in a Western blot format in which samples of various zvegf3 proteins were reduced and electrophoresed on a polyacrylamide gel. The proteins used were: recombinant human zvegf3 growth factor domain, recombinant human zvegf3 full-length, and recombinant human zvegf3 full-length fused to MBP, each at concentrations of 13.9, 41.7, and 125 ng/lane; and conditioned media from HaCat cells expressing full-length human zvegf3. The electrophoresed proteins were then transferred to a nitrocellulose membrane, rinsed, and blocked by overnight incubation in buffer containing 2.5% non-fat dry milk. The primary antibody (E2243 antisera) was diluted to 300 ng/ml and added to the nitrocellulose blot, which was then incubated for 1 hour at room temperature with shaking. The blot was then rinsed, secondary antibody (anti-rabbit IgG conjugated to horseradish peroxidase) was added, and the blot was incubated for 1 hour at room temperature with shaking. The blot was then rinsed, developed with commercially available substrates, and exposed to film for 10 seconds.

6. The Western blot showed that the E2243 antisera recognized all samples of full-length zvegf3 (fused and unfused) and the zvegf3 in the conditioned media. The antisera did not recognize any of the samples of isolated zvegf3 growth factor domain.

7. In summary, antisera raised against a full-length recombinant human zvegf3 fusion protein bound to fused and unfused full-length human zvegf3, but did not bind to isolated human zvegf3 growth factor domain, when tested in a Western blot format.

I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further, that these statements were made with the knowledge that the making of willfully false statements and the like is punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and may jeopardize the validity of any patent issuing from this patent application.

June 3, 2003

Date

Henry Francis Pelto III